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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Task 1: Engineer Laminin-collagen IV-entactin-perlecan (LCEP) binding domains as fusions to the growth and neurotrophic factors ('protein factors'). Generate LCEP composite fiber scaffolds incorporating protein factors exhibiting either constant surface density or longitudinal density gradients. Match fiber stiffness with that of autograft (G*~1 MPa). Characterize the bioactivity of scaffold-bound protein factors using cell signaling assays (0-15 months); performance site: USMA. *Task 2*: Evaluate each protein factor (singly & in selected combinations) in the organotypic model system for preferential sensory or motor axon extension. Use confocal microscopy to track axon extension of labeled sensory or motor neurons from spinal cord slices (motor) or dorsal root ganglia (DRG) (sensory). 20 Thy1-YFP mice will be used for this task and subtasks. (12-24 mo.); sites: JHU & USMA *Task 3*: Evaluate top performing compositions from *Task 2* in a rat femoral nerve repair model in which sensory and motor axons intermingle proximally and are segregated into discrete cutaneous and muscle branches distally. 80 Sprague-Dawley rats will be used for this task and subtasks. (24-36 mo.); work performed at JHU.

15. SUBJECT TERMS nothing listed

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INTRODUCTION:

In this project we will develop a new type of nerve guide that improves the rate of nerve regeneration by directing subsets of axons to the peripheral pathways that best support their growth. We will evaluate a panel of growth and neurotrophic factors functionalized onto 3D fiber scaffolds for their ability to selectively promote the extension of sensory or motor axons. Fibers that preferentially guide either axon type will be used to make multifunctional nerve guides that can sort a mixed population of regenerating axons into sensory and motor tributaries. By guiding axons to appropriate pathways, this novel nerve guide will improve clinical outcomes by enhancing both regeneration speed and the functional specificity of end organ connections.

KEYWORDS: nerve, regeneration, axon, neural, interface, device, conduit, guide, repair, neuron, prosthetic, neuroprosthetic, peripheral

OVERALL PROJECT SUMMARY:

Task 1: Engineer Laminin-collagen IV-entactin-perlecan (LCEP) binding domains as fusions to the growth and neurotrophic factors ('protein factors'). Generate LCEP composite fiber scaffolds incorporating protein factors exhibiting either constant surface density or longitudinal density gradients. Characterize the bioactivity of scaffold-bound protein factors using cell signaling assays (0-15 months); performance site: NCI / USMA.

We have continued to work task 1 in the SOW. We hired a staff neuroscientist Dr. Joseph Loverde to lead several aspects of the project. Previously Dr. Loverde was a post-doctoral associate in the laboratory of Dr. Bryan Pfister where he pioneered the use of axon stretch growth to study mechanisms of nerve regeneration.

Since Dr. Loverde's arrival in September 2014, we have started fiber fabrication and are working on wet-extrusion methods. This work has focused on the fabrication of sample fibers in small quantities using collagen as a base. As risk mitigation, we have also initiated discussions with the electrospinning group at USMA (West Point) to explore the use of electrospinning for this project. We have made progress in completing the design on plasmid design software for all of our constructs. Our work during the previous reporting period focused on completing the design of the recombinant expression cassettes for each of the protein factors listed in the proposal, namely: Brain derived neurotrophic factor (BDNF), Nerve growth factor (NGF), Insulin-like growth factor 1 (IGF-1), Hepatocyte growth factor (HGF), and Pleitotrophin (PTN). We are using a gene synthesis service to make our sequences de novo. Variants to our sequences include tandem heparin binding peptide (HBP)2 and / or tandem laminin binding peptide (LBP)2.

We were successful in gaining ACURO approval for animal work which will take place in Aim 3 of the project.

KEY RESEARCH ACCOMPLISHMENTS:

- Established a system of color-coded mixed nerve tracking using GFP and RFP expressing motor and sensory neurons (Figure 1 below).

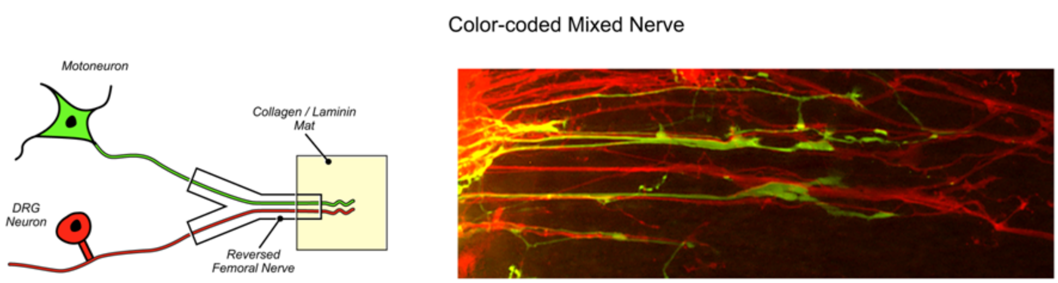

Figure 1 Motor (green) and sensory (red) axons enter the muscle and cutaneous branches of a reversed segment of femoral nerve and progressively intermingle. When this nerve is transected and axons grow out onto a collagen/laminin mat, **sensory axons grow out first and trap motor axons by adhesion**.

- Completed the genetic design of 20 variants for 5 neurotrophic factors listed in the SOW. This design includes the expression cassette design for E. coli based expression. The variants include dual binding domains for heparin sulfate and / or laminin. 25-Mar-2014
- Completed interviews for candidates for the lead Post-doc/Scientist position; anticipate hiring in early June to coincide with LTC Alvarez' reassignment to West Point, NY. 26-Mar-2014
- Initiated a collaboration with the University of Texas at Arlington to assist in evaluating our in vitro axon segregation model. Mar-2014
- Identified electrospinning capability at USMA (West Point, NY) to provide a backup method of producing aligned fibers as stated in the SOW. 13-Dec-2013
- Identified a potential method of characterizing biological responses using a microplate reader that may enable much faster processing than the use of a core facility as is currently proposed. If purchased, the microplate reader would represent a no-cost change to current budget allocation but would result in a significant time savings for the project overall.

CONCLUSION:

Our work focuses on the physical separation and outgrowth of sensory from motor neurons. This physical sorting and separation is a key problem in nerve regeneration. We have identified neurotrophic factors that guide the preferential extension of motor vs sensory neurons. This differential effect forms the basis of separation by type.

An additional application of our work beyond nerve regeneration is in the development of neuroprosthetic interfaces. The physical separation of motor from sensory axons permits the coupling of either myoelectric or sensory input electrode at physically separated sites. This has applications in giving prosthetics the sense of touch and in vastly enhancing the quality of motor function in the affected limb.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS: Nothing to Report

INVENTIONS, PATENTS AND LICENSES: Nothing to report.

REPORTABLE OUTCOMES:

- Based on #2 below we have obtained funding from DARPA for a complementary undergraduate project in nerve regeneration.
- We have identified a new high-impact application of our work in the field of motor-sensory prosthetics. This application came to light during discussions with DARPA-funded investigators at the University of Texas at Arlington. Although the intent of our current work is to restore function to injured limbs the results of our work will have direct applicability to neural interfaces for advanced prosthetics that require separation of sensory and motor axons.

OTHER ACHIEVEMENTS: Nothing to report.

REFERENCES: Nothing to report.

APPENDICES: Nothing to report.